

CLAIMS

What is claimed is:

1. A method for the expression of a coding region of interest in a C1 metabolizing bacteria comprising:

- 5 a) providing a transformed C1 metabolizing bacterial cell having a chimeric gene comprising;
- 1) a promoter region of a gene selected from the group consisting of: a *nrtA* gene and a *glnB* gene; and
- 2) a coding region of interest expressible in a C1 metabolizing bacteria;
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wherein the promoter region is operably linked to a coding region of interest; and

- b) growing the transformed C1 metabolizing bacteria cell of step (a) in the presence of nitrate wherein the chimeric gene is expressed.
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2. A method for the expression of a coding region of interest in a C1 metabolizing bacteria comprising:

- a) providing a transformed C1 metabolizing bacterial cell having a chimeric gene comprising;
- 20 1) a promoter region of a *glyoxII* gene; and
- 2) a coding region of interest expressible in a C1 metabolizing bacteria;

wherein the promoter region is operably linked to a coding region of interest; and

- 25 b) growing the transformed C1 metabolizing bacteria cell of step (a) at a pH of about 5.5 wherein the chimeric gene is expressed.

3. A method for the expression of a coding region of interest in a C1 metabolizing bacteria comprising:

- 30 a) providing a transformed C1 metabolizing bacterial cell having a chimeric gene comprising;
- 1) a promoter region of a *htpG* gene; and
- 2) a coding region of interest expressible in a C1 metabolizing bacteria;

35 wherein the promoter region is operably linked to a coding region of interest; and

- b) growing the transformed C1 metabolizing bacteria cell of step (a) at a temperature suitable for induction of the promoter region wherein the chimeric gene is expressed.
4. A method for the expression of a coding region of interest in a C1 metabolizing bacteria comprising:
- a) providing a transformed C1 metabolizing bacterial cell having a chimeric gene comprising;
- 1) a promoter region of a gene selected from the group consisting of: a *moxF* gene and a *hps* gene; and
- 2) a coding region of interest expressible in a C1 metabolizing bacteria;
- wherein the promoter region is operably linked to a coding region of interest; and
- b) growing the transformed C1 metabolizing bacteria cell of step (a) in the presence of a C1 carbon source selected from the group consisting of methane and methanol wherein the chimeric gene of step (a) is expressed.
5. A method according to any of Claims 1-4 wherein the C1 metabolizing bacterial host cell is selected from the group consisting of methanotrophs and methylotrophs.
6. A method according to Claim 5 wherein the C1 metabolizing bacterial host cell is a methylotroph selected from the group consisting of *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylocystis*, *Methylomicrobium*, *Methanomonas*, *Methylophilus*, *Methylobacillus*, *Methylobacterium*, *Hyphomicrobium*, *Xanthobacter*, *Bacillus*, *Paracoccus*, *Nocardia*, *Arthrobacter*, *Rhodopseudomonas*, and *Pseudomonas*.
7. A method according to Claim 1 wherein the promoter region has the nucleic acid sequence selected from the group consisting of SEQ ID NO:3 and SEQ ID NO:6.
8. A method according to Claim 2 wherein the promoter region has the nucleic acid sequence as set forth in SEQ ID NO:9.
9. A method according to Claim 3 wherein the promoter region has the nucleic acid sequence as set forth in SEQ ID NO:12.
10. A method according to Claim 3 wherein the temperature suitable for induction of the promoter region is selected from the group consisting of:

- a) 41-42°C wherein the C1 metabolizing bacteria is mesophilic; and
 - b) 47-50°C wherein the C1 metabolizing bacteria, is thermophilic
- 5 11. A method according to Claim 4 wherein the nucleic acid fragment comprising the promoter region has the nucleic acid sequence selected from the group consisting of SEQ ID NO:15, and 18.
12. A method according to Claim 1 wherein the concentration of nitrate is from about 5mM to about 15mM.
- 10 13. The method according to any one of Claims 1 – 4 wherein the coding region of interest is selected from the group consisting of genes encoding: transaldolase, fructose bisphosphate aldolase, keto deoxy phosphogluconate aldolase, phosphoglucomutase, glucose-6-phosphate isomerase, phosphofructokinase, 6-phosphogluconate dehydratase, 6-
15 phosphogluconate-6-phosphate-1 dehydrogenase, *dxs*, *dxr*, *ispA*, *ispD*, *ispE*, *ispF*, *crtE*, *crtX*, *crtY*, *crtl*, *crtB*, *crtZ*, *crtD*, *crtO*, *crtW*, genes encoding limonene synthase, *ugp*, *gumD*, *wza*, *espB*, *espM*, *waaE*, *espV*, *gumH*, genes encoding glycosyltransferase genes, *aroG*, *aroB*, *aroQ*, *aroE*, *aroK*, 5-enolpyruvylshikimate-3-phosphate synthase, *aroC*, *trpE*, *trpD*, *trpC*,
20 *trpB*, *pheA*, *tyrAc*, *pds*, *phaC*, *phaE*, *efe*, *pdc*, *adh*, pinene synthase, bornyl synthase, phellandrene synthase, cineole synthase, sabinene synthase, and taxadiene synthase.
14. A method for the production of zeaxanthin comprising:
 - a) providing a transformed C1 metabolizing host cell
25 comprising:
 - 1) suitable levels of b-Carotene; and
 - 2) a chimeric gene comprising the promoter region of the *hps* gene operably linked to a coding region encoding β -carotene hydroxylase; and
 - 30 (b) contacting the host cell of step (a) under suitable growth conditions with an effective amount of a C1 carbon substrate whereby an zeaxanthin is produced.
15. An isolated nucleic acid molecule encoding a nitrate inducible gene selected from the group consisting of:
 - 35 (a) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:5;

- (b) an isolated nucleic acid molecule that hybridizes with (a) under stringent conditions and is washed with 0.1X SSC, 0.1% SDS, 65°C; or
an isolated nucleic acid molecule that is complementary to (a),
5 or (b).
16. The isolated nucleic acid molecule of Claim 15 selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:4.
17. A polypeptide encoded by the isolated nucleic acid molecule of Claim 15.
- 10 18. The polypeptide of Claim 17 selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:5.
19. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a nrtA enzyme of at least 464 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment
15 when compared to a polypeptide having the sequence as set forth in SEQ ID NO:2;
or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
20. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a glnB enzyme of at least 112 amino acids that has at least 76% identity based on the Smith-Waterman method of alignment
20 when compared to a polypeptide having the sequence as set forth in SEQ ID NO:5;
or a second nucleotide sequence comprising the complement
25 of the first nucleotide sequence.
21. An isolated nucleic acid molecule encoding a pH inducible gene selected from the group consisting of:
- a) an isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO:8;
- 30 b) an isolated nucleic acid molecule that hybridizes with (a) under stringent conditions and is washed with 0.1X SSC, 0.1% SDS, 65°C; or
an isolated nucleic acid molecule that is complementary to (a),
or (b).
- 35 22. The isolated nucleic acid molecule of Claim 21 as set forth in SEQ ID NO:7.
23. A polypeptide encoded by the isolated nucleic acid molecule of Claim 21.

24. The polypeptide of Claim 23 having the amino acid sequence as set forth in SEQ ID NO:8.

25. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a glyoxII enzyme of at least 231 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:8;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

26. An isolated nucleic acid molecule encoding a temperature inducible gene selected from the group consisting of:

- a) an isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO:11;
- b) an isolated nucleic acid molecule that hybridizes with (a) under stringent conditions and is washed with 0.1X SSC, 0.1% SDS, 65°C; or an isolated nucleic acid molecule that is complementary to (a), or (b).

27. The isolated nucleic acid molecule of Claim 26 as set forth in SEQ ID NO:10.

28. A polypeptide encoded by the isolated nucleic acid molecule of Claim 26.

29. The polypeptide of Claim 28 having the amino acid sequence as set forth in SEQ ID NO:11.

30. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a htpG enzyme of at least 644 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:11;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

31. An isolated nucleic acid molecule encoding a methane or methanol inducible gene selected from the group consisting of:

- (a) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ ID NO:14, and 17;
- (b) an isolated nucleic acid molecule that hybridizes with (a) under stringent conditions and is washed with 0.1X SSC,

0.1% SDS, 65°C; or an isolated nucleic acid molecule that is complementary to (a), or (b).

32. The isolated nucleic acid molecule of Claim 31 selected from the group consisting of SEQ ID NO:13, and 16.

5 33. A polypeptide encoded by the isolated nucleic acid molecule of Claim 31.

34. The polypeptide of Claim 33 selected from the group consisting of SEQ ID NO:14, and 17.

10 35. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a moxF enzyme of at least 89 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:14;

15 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

36. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a hps enzyme of at least 215 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:17;

20 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

37. A promoter region responsive to the presence of nitrate having the nucleic acid sequence selected from the group consisting of SEQ ID NO:3 and SEQ ID NO:6.

38. A promoter region responsive to acidic pH having the nucleic acid sequence as set forth in SEQ ID NO:9.

39. A promoter region responsive to elevated temperatures having the nucleic acid sequence as set forth in SEQ ID NO:12.

30 40. A promoter region highly expressed in the presence of methane or methanol having the nucleic acid sequence selected from the group consisting of SEQ ID NO:15, and SEQ ID NO:18.